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PATENT
Attorney Docket No. 019957-013821US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Shawn DeFrees *et al.*

Application No.: not yet assigned

Filed: herewith

For: LOW COST MANUFACTURE OF OLIGOSACCHARIDES

Examiner: Christian L. Fronda
(parent)

Art Unit: 1652

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

In the Specification:

At page 4 line 11, change "Figure 5" to --Figure 6--.

At page 4 line 18, change "Figure 6A and 6B" to --Figure 5A and 5B--.

At page 4 line 22, change "Figure 6A" to --Figure 5A--.

At page 5 line 3, change "Figure 6B" to --Figure 5B--.

At page 56 line 22, change "*Glycobiology*3" to --*Glycobiology* 3--.

In the Claims:

Please cancel claims 1-71 without prejudice.

Please add the following new claims 72-105:

72. A method for synthesizing a polysaccharide backbone for heparin, heparan sulfate and related compounds, the method comprising contacting an acceptor saccharide that comprises a terminal glucuronic acid or GlcNAc residue with a reaction mixture that comprises:

a microorganism or plant cell that comprises:

a) an enzymatic system for forming UDP-GlcNAc; and

b) a recombinant GlcNAc transferase that catalyzes the transfer of GlcNAc from the UDP-GlcNAc to a terminal glucuronic acid on the acceptor saccharide to produce an acceptor saccharide that comprises a terminal GlcNAc residue; and

a microorganism or plant cell that comprises:

a) an enzymatic system for forming UDP-glucuronic acid; and

b) a recombinant glucuronic acid transferase that catalyzes the transfer of glucuronic acid from the UDP-glucuronic acid to a terminal GlcNAc residue on the acceptor saccharide to produce an acceptor saccharide that comprises a terminal glucuronic acid residue; and

allowing the reaction to proceed until the polysaccharide backbone is synthesized.

73. The method of claim **72**, wherein the reaction mixture comprises a single cell type that comprises:

a) enzymatic systems for forming UDP-GlcNAc and UDP-glucuronic acid;

b) a recombinant GlcNAc transferase; and

c) a recombinant glucuronic acid transferase.

74. The method of claim **72**, wherein the enzymatic system for forming UDP-GlcNAc and the recombinant GlcNAc transferase are in a first cell type, and the enzymatic system for forming UDP-glucuronic acid and the recombinant glucuronic acid transferase are in a second cell type.

75. The method of claim **72**, wherein either or both of the enzymatic systems for producing UDP-GlcNAc and UDP-glucuronic acid comprises a full or partial sugar nucleotide regeneration cycle.

76. A method for synthesizing heparin, heparan sulfate and related compounds, the method comprising contacting a heparan polysaccharide backbone with a reaction mixture that comprises a microorganism or plant cell which comprises:

a) an enzymatic system for forming PAPS; and

b) a recombinant sulfotransferase which catalyzes the transfer of a sulfate from the PAPS to the heparan polysaccharide backbone to produce an N-sulfated polysaccharide.

77. The method of claim **76**, wherein the enzymatic system for forming PAPS comprises a PAPS cycle.

78. The method of claim **76**, wherein the method further comprises contacting the N-sulfated polysaccharide with a glucuronic acid 5'-epimerase to convert one or more glucuronic acid residues in the polysaccharide backbone to iduronic acid.

79. The method of claim **78**, wherein the glucuronic acid 5'-epimerase is expressed by a cell present in the reaction mixture that comprises a gene that encodes glucuronic acid 5'-epimerase.

80. The method of claim **78**, wherein the method further comprises contacting the iduronic acid-containing N-sulfated polysaccharide with one or more *O*-sulfotransferases to form heparan sulfate.

81. The method of claim **80**, wherein the *O*-sulfotransferase is expressed by a cell present in the reaction mixture that comprises a gene that encodes the *O*-sulfotransferase.

82. The method of claim **81**, wherein the cell that expresses the *O*-sulfotransferase further comprises an enzymatic system for forming PAPS.

83. The method of claim **76**, wherein the heparan polysaccharide backbone is obtained by a method that comprises:

contacting an acceptor saccharide that comprises a terminal glucuronic acid or GlcNAc residue with a reaction mixture that comprises:

1) a microorganism or plant cell that comprises:
   a) an enzymatic system for forming UDP-GlcNAc; and
   b) a recombinant GlcNAc transferase that catalyzes the transfer of GlcNAc from the UDP-GlcNAc to a terminal glucuronic acid on the acceptor saccharide to produce an acceptor saccharide that comprises a terminal GlcNAc residue; and
2) a microorganism or plant cell that comprises:
   a) an enzymatic system for forming UDP-glucuronic acid; and
   b) a recombinant glucuronic acid transferase that catalyzes the transfer of glucuronic acid from the UDP-glucuronic acid to a terminal GlcNAc residue on the acceptor saccharide to produce an acceptor saccharide that comprises a terminal glucuronic acid residue; and

allowing the reaction to proceed until the polysaccharide backbone is synthesized.

84. The method of claim **72**, wherein the method further comprises sulfating a heparan polysaccharide backbone to form an N-sulfated heparan polysaccharide backbone.

85. The method of claim **84**, wherein the method further comprises contacting the N-sulfated polysaccharide with a glucuronic acid 5'-epimerase to convert one or more glucuronic acid residues in the polysaccharide backbone to iduronic acid.

86. The method of claim **85**, wherein the method further comprises contacting the iduronic acid-containing N-sulfated polysaccharide with one or more *O*-sulfotransferases to form heparan sulfate.

87. The method of claim **53**, wherein the method further comprises contacting the microorganism or plant cell with a second cell type that produces a nucleotide that is used as a substrate for the enzymatic system for forming the nucleotide sugar.

88. The method of claim **87**, wherein the second cell type comprises an exogenous gene that encodes a nucleotide synthetase polypeptide that catalyzes the synthesis of the nucleotide.

89. The method of claim **88**, wherein the microorganism or plant cell comprises exogenous genes that encode a) a fusion protein that comprises a polypeptide having 3'-sialyltransferase activity and a polypeptide that has CMP-sialic acid synthetase activity; and b) enzymes that catalyze the synthesis of sialic acid from GlcNAc;

and the second cell type comprises an exogenous gene that encodes CMP-synthetase.

90. The method of claim **88**, wherein the microorganism or plant cell is *E. coli* and the second cell type is yeast or *Corynebacterium*.

91. The method of claim **87**, wherein the microorganism or plant cell produces a second recombinant glycosyltransferase that catalyzes the transfer of a sugar from the nucleotide sugar to the product saccharide to form a further glycosylated product saccharide.

92. The method of claim **91**, wherein the nucleotide sugar is UDP-Gal, the first recombinant glycosyltransferase is an β1,4-galactosyltransferase and the second recombinant glycosyltransferase is an α1,3-galactosyltransferase.

93. The method of claim **92**, wherein the acceptor saccharide is Glc(R)β-O-R^1, wherein R^1 is -$(CH_2)_n$-COX; X is selected from the group consisting of OH, OR^2, -$NHNH_2$, R is OH or NAc; R^2 is a hydrogen, a saccharide, an oligosaccharide or an aglycon group having at least one carbon atom, and n is an integer from 2 to 18.

94. The method of claim **92**, wherein the UDP-Gal is generated by enzymes that are expressed from exogenous genes that encode UDP-Gal 4' epimerase and UDP-Glc pyrophosphorylase.

95. The method of claim **53**, wherein the microorganism or plant cell further comprises: a) an enzymatic system for producing at least a second nucleotide sugar, and b) at least a second recombinant glycosyltransferase that catalyzes transfer of a sugar from the second nucleotide sugar to the product sugar.

96. The method of claim **95**, wherein:

the first recombinant glycosyltransferase is a GlcNAc transferase and the first nucleotide sugar is UDP-GlcNAc; and

the second recombinant glycosyltransferase is a galactosyltransferase and the second nucleotide sugar is UDP-galactose.

97. The method of claim **96**, wherein the method forms lacto-N-neotetraose (LNnT).

98. The method of claim **53**, wherein the method further comprises contacting the product saccharide with at least a second type of cell that produces a) a second nucleotide sugar, and b) a second recombinant glycosyltransferase that catalyzes the transfer of the sugar from the second nucleotide sugar to the product saccharide.

99. The method of claim **98**, wherein the second type of cell is a plant or microorganism cell.

100. The method of claim **98**, wherein one cell type comprises a galactosyltransferase and another cell type comprises a GalNAc transferase.

101. The method of claim **98**, wherein:

one cell type comprises a recombinant β1,4-GalNAc transferase, a recombinant β1,4-Gal transferase, UDP-GalNAc and UDP-Gal; and

another cell type comprises a recombinant α2,3-sialyltransferase and CMP-sialic acid.

102. The method of claim **101**, wherein the CMP-sialic acid is produced from CTP and GlcNAc by an enzymatic system that includes recombinant enzymes CMP-sialic acid synthetase, GlcNAc epimerase, NeuAc aldolase, and CMP-synthetase.

103. The method of claim **101**, wherein the acceptor saccharide is lactosylceramide or lyso-lactosylceramide and the product saccharide is ganglioside GM_2.

104. The method of claim **101**, wherein one cell type further comprises a recombinant α2,8-sialyltransferase.

105. The method of claim **104**, wherein the acceptor is lactosylceramide or lyso-lactosylceramide and the product saccharide is GD_2.

REMARKS

Status of the Application

Claims 72-105 are pending with entry of this amendment.

The Amendments

The amendments to the specification at page 4 correct an error in the figure references.

New claims 72-105 find support in the specification and in the originally filed claims as follows:

New claim 72 finds support at, for example, Figure 7; page 5, lines 11-30; and page 56, lines 14-15.

New claim 73 finds support at, for example, Figure 7 and page 5, lines 11-30.

New claim 74 finds support at, for example, originally filed claim 31, and page 48, lines 1-2.

New claim 75 finds support at, for example, page 26, line 16 bridging to page 27, line 7.

New claim 76 finds support at, for example, Figure 7; page 5, lines 11-30; page 46, lines 27 bridging to page 47, line 9; and page 57, lines 4-11.

New claim 77 finds support at, for example, Figure 6 and page 4, lines 11-17.

New claims 78-86 find support at, for example, page 5 lines 11-30, page 22 line 26 to page 23 line 8, page 46 line 27 to page 47 line 11, page 57 lines 5-11, originally filed claim 50, and Figures 6 and 7.

New claim 87 finds support at, for example, originally filed claim 20 and page 48, lines 1-2.

New claim 88 finds support at, for example, originally filed claim 21 and page 18, lines 6-8.

New claim 89 finds support at, for example, originally filed claim 22 and Figure 5B and page 5, lines 3-10.

New claim 90 finds support at, for example, originally filed claim 23, Figure 5B and page 5, lines 3-10.

New claim 91 finds support at, for example, originally filed claim 24 and page 29, lines 5-8.

New claim 92 finds support at, for example, originally filed claim 25 and Figure 11.

New claim 93 finds support at, for example, originally filed claim 26.

New claim 94 finds support at, for example, originally filed claim 27 and Figure 11B.

New claim 95 finds support at, for example, originally filed claim 28 and page 29, lines 5-8.

New claim 96 finds support at, for example, originally filed claim 29 and Figure 2.

New claim 97 finds support at, for example, originally filed claim 30 and Figure 2.

New claim 98 finds support at, for example, originally filed claim 31 and page 7, lines 22-23 and page 48, lines 1-2.

New claim 99 finds support at, for example, originally filed claim 2 and page 7, line 19.

New claim 100 finds support at, for example, originally filed claim 32.

New claim 101 finds support at, for example, originally filed claim 33 and Figure 10.

New claim 102 finds support at, for example, originally filed claim 34 and Figure 8.

New claim 103 finds support at, for example, originally filed claim 35 and Figure 8.

New claim 104 finds support at, for example, originally filed claim 36 and Figure 9.

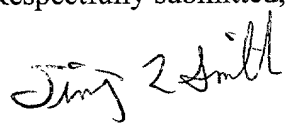
New claim 105 finds support at, for example, originally filed claim 37 and Figure 9.

CONCLUSION

In view of the foregoing, Applicants believe that all claims now pending in this application are in condition for examination.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned attorney at (415) 576-0200.

Respectfully submitted,



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United States Patent Application

LOW COST MANUFACTURE OF OLIGOSACCHARIDES

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